D. A PROBABILISTIC MODEL FOR THE ASSAY AND CERTIFICATION OF STERILITY

(See Section IV. C. for a discussion of the microbiological aspects of assay and certification of sterility.)

E. MICROBIOLOGICAL CONSIDERATIONS OF EASL AND SADL FACILITIES

1. Introduction

Several standards have been published on methods for control of total particulate contamination within the intramural environments of cleanrooms. In August 1964, NASA presented the first version of a standard to control the microbial contamination within facilities involved in the assembly and/or test of interplanetary spacecraft. A standard (known as the Interim Requirements for Bioclean Facilities) has been revised several times and the revised version that is to be implemented in the JPL EASL Facility is appended. More recently, NASA has issued a requirement that assembly of spacecraft hardware is to be conducted in a vertical laminar flow cleanroom meeting Class 100 specifications as stated in Federal Standard No. 209 in lieu of the "bioclean assembly requirements". This laminar flow concept is a judgment on the part of NASA, and the EASL Facility is being constructed and operated to evaluate both the laminar down flow and the bioclean room requirements.

The Interim Requirements for Bioclean Facilities is reproduced in Appendix B.

2. Design and/or Construction of Facilities to Implement the Interim Requirements for Bioclean Facilities

The Jet Propulsion Laboratory has two facilities in construction or design to implement the NASA Interim Requirements for Bioclean Facilities. These two facilities have been designated as: (1) an Experimental Assembly and Sterilization Laboratory (Project EASL); and (2) the Sterile Assembly Development Laboratory (Project SADL). A third facility, the Assembly Test and Sterilization Facility (AT & SF) is presently being considered. However only the first two projects, EASL and SADL, are active at present and discussion shall be confined to these two facilities.

a. Project EASL

This project will result in the development of an Experimental Assembly and Sterilization Laboratory. Construction of Project EASL began 15 March 1965, in a portion of the southwest corner of the low bay area in JPL, Building 233. The project is presently on schedule, with completion being forecast for early June and the facility is expected to be certified operational by 1 July 1965. The purpose of this facility is to conduct an investigation into some of the procedures and techniques for the assembly and/or test of spacecraft in a facility operating under the full implementation of the NASA Interim Requirements for Bioclean Facilities (see Appendix B). Assembly and test shall be at the component and subsystem level. Simultaneously, a duplication of the work being done in the EASL shall be conducted under "non-bioclean", or the routine laboratory conditions and procedures. Scheduled microbiological assays of the hardware and assembly environment shall be conducted throughout all operational stages of both the bioclean and non-bioclean assemblies. Finally, both the non-bioclean and the bioclean assemblies shall be exposed to dry heat for 24 hr (135°C) in a nitrogen atmosphere and then assayed for sterility. The cost in time and money, as well as the ability to satisfy the sterilization requirement will be compared, contrasted and recorded. Functional reliability of the assembled units will also be checked. The microbiological monitoring of the intramural environment of EASL also completes the range of controlled environments being qualitatively and quantitatively surveyed by the JPL sterilization group for the degree of microbial contamination present. This survey ranges for an area of little environmental control (JPL Building 179, Spacecraft Assembly Facility) through Class II, III and IV clean rooms (JPL/Douglas Contract No. 950920) to the final evaluation of the levels of microbial contamination occurring in the air and on surfaces within a vertical and a horizontal laminar air flow clean room (Project EASL).

b. Project SADL

Project SADL will be a Sterile Assembly Development Laboratory to evaluate the technology and procedures for Voyager hardware at the required levels of assembly and test under the full implementation of the NASA Interim Requirements for Bioclean Facilities (see Appendix B). Project SADL will be a microbiologically sufficient facility, including a complete microbiology laboratory for conducting all the microbiological assays necessary for monitoring all of the SADL operations, subcontractors and vendors. The complex nature of the SADL facility operations, the monitoring of vendors, and the increased number of operating personnel, may negate extrapolation of observations from the EASL to a large scale operation as designed for Project SADL.

The SADL facility will also operate as a training facility for technical and supervisory personnel. Within this facility trainees can become aware of and practice the most advanced state-of-the-art procedures and techniques for assembly and test under bioclean conditions. The SADL facility will also serve as the "pilot plant" for the development and implementation of special handling equipment, fixtures, operational equipment and any other necessary support equipment required for bioclean assembly.

The SADL facility will provide JPL with the capability to conduct other functions such as:

1) Microbiologically certify and monitor the quality of subcontractor and vendor items.

2) Microbiological monitoring throughout all stages of assembly and test.

3) Development and establishment of personnel procedures to control dissemination of microorganisms.

4) Evaluation of the implementation of the NASA Interim Requirements for Bioclean Facilities on all elements of applicable hardware design, assembly and test. Perhaps leading to modification and/or optimization of these requirements with the overall operational plan of assembly and/or testing.

5) Collect process and evaluate the results of the microbiological and visual assays, parametric measurements and terminal sterilization activities. All essential in providing the documentation necessary for certification of spacecraft sterility.

6) In conjunction with 5) above evaluate the adequacy and accuracy of the math model and probability model.

F. DATA HANDLING

The amount of data to be handled on any mission which must meet the requirement of planetary quarantine will, of necessity, be quite large. Proof must be formulated that the planetary quarantine requirements have been met.

Although we are unable at this time to stipulate detailed requirements or methods, this must be recognized as a significant problem area.

This Engineering Document will be updated as information relating to data handling becomes available.

SECTION IV

MICROBIOLOGY

A. MICROBIAL CONTAMINATION

1. Introduction

Microbial contamination may be defined as the presence of unwanted living microorganisms in a given environment. In its strictest sense, microbial contamination could be considered to include all sorts of microscopic animals and plants or parts thereof; e.g., bacteria, fungi, viruses, protozoa, algae, pollen, etc. However, in a practical sense only two groups of microorganisms, the bacteria and the fungi, are generally considered as the indices of microbial contamination. For the purposes of this discussion, the subject matter will be certain aspects of the basic biology of these two groups and the problems related to their destruction (sterilization).

A third group of organisms which may be of significance in microbial contamination problems is the viruses. There is very little information concerning the behavior of viruses as contaminants. Viruses do not presently fall within the scope of the NASA policy thus they will not be discussed as contaminating agents, except to indicate them as a possible problem area.

2. Bacteria

Bacteria are characterized as single cell plants; the cells being usually microscopic and occasionally ultramicroscopic. The size of bacterial cells may vary from 0.1 micron (μ) in diameter to several microns in length; however, the majority of the commonly encountered organisms measure approximately 0.5 μ in diameter to perhaps 2 to 3 μ in length for cells with elongated forms ($1\,\mu = 10^{-3}$ mm or 1/25,400 in.).

Some bacteria will have the capability to form spores (sporulate). The bacterial spore is extremely resistant to sterilization treatments and therefore represents a major problem in the control of microbial contamination.

3. Fungi

The fungi, like the bacteria, are members of the plant kingdom although they have no stems, roots, or leaves. The term fungi has no exact definition; it is a general and convenient term applied to the growths of black, white, green, or yellowish coloration that are

observed on stale bread, on the tops of jams and jellies, or on books and leather goods in the summertime when the humidity is high. However, these are only some common examples. A majority of the fungi are saprophytic and, therefore, are commonly associated with decaying vegetative matter; others are parasitic on a broad variety of green plants.

4. Distribution of Microorganisms

Bacteria and fungi are widely distributed over the Earth's surface — in the soil, atmosphere, fresh water, sea water, and in and on the bodies of plants and animals. They are so ubiquitous that few places in the Earth's environment are free of their presence. The microbial population far exceeds in numbers the total population of all plants and animals on Earth. Because of the ubiquitous nature of the two groups, they are predominant sources of medical and industrial problems, where their presence may not only be undesirable but in some instances disasterous.

5. Resistance of Microorganisms to Environmental Conditions

The microbial contamination level of a given environment is dependent to some extent upon the relative resistance of the individual microorganisms to the conditions of that environment. The most resistant forms of bacteria and fungi are the spore forms. It has already been mentioned that the formation of spores by fungi is a part of their reproductive cycle. Certain species of bacteria produce spores within their individual cells. This type of spore is called an endospore. However, the bacterial spore is not a reproductive form as in the fungi, but rather it is a resistant form that enables the cell to survive many of the destructive forces in its environment. Certain bacterial cells are readily killed by drying and desiccation while others that withstand the drying are killed by slightly elevated conditions of temperature and humidity. For example, certain members of the Proteus group suffer little effect from exposure to 11 percent relative humidity at 25°C but the number of recoverable cells is decreased markedly at 53 percent relative humidity at the same temperature. Cells of the different groups of bacteria and molds exhibit varying degrees of resistance to physical and chemical agents such as ultraviolet light and toxic chemical vapors or liquids. The resistance of a cell may be influenced by the immediate physical environment of the cell. Thus, the cell located within the mass of a group of cells may be protected from some external influences. Similarly, cells may be protected by a variety of materials which may be of organic or inorganic origin. Such variations in resistivity will cause fluctuations in the observed index of microbial contamination and also makes it apparent that the application of contamination control must be carefully scrutinized.

6. Nature of Microbial Contamination

Microbial contamination exists in the air and on surfaces within any given environment. It is doubtful that many of those contaminants occur as individual free cells. Most of them

are associated with carriers or airborne particulate matter such as dust and lint. The microbial cells associated with a carrier may occur individually or in clumps. In those areas where a number of persons may be present, airborne of contamination may be in the form of droplet nuclei. It is generally assumed that the higher the total particulate contamination level the higher will be the microbial contamination although it is not necessarily true for all conditions. It is quite possible to have a constant particulate contamination level with a varying viable particle level. In general, however, those factors which influence particle distribution and sedimentation rates will influence microbial contamination levels in the air and on surfaces in some positive proportion.

Surfaces which are handled by people may be contaminated by individual cells, clumps of cells or microbial-bearing particles. The microbial contamination level of those surfaces handled by people with bare hands will generally be much higher than samples contaminated by sedimentation.

B. REDUCTION OF MICROBIAL CONTAMINATION

1. Definitions

a. Sterilization

The term sterility is defined as the complete destruction or removal of all life and refers to an absolute condition. Thus, used in its proper sense, there is no such thing as "almost" or "nearly" sterile, since the presence of only one microorganism creates an unsterile condition. Sterilization refers to the process or the methods for producing a sterile condition. The two terms, sterility and sterilization, are often used loosely and erroneously in reference to conditions short of sterility. The medical field and the food industry often use the terms in relation to the destruction of certain groups of harmful or undesirable microorganisms and not in reference to complete destruction of life. In the food industry the terms are used primarily in relation to destruction of spoilage organisms and in the medical sense they usually refer to distinction of pathogenic (disease-producing) organisms. The important point to be recognized is that the terms are not relative and their use as such is improper and often leads to a great deal of misunderstanding.

b. Decontamination

A term and process which is frequently confused with sterilization is microbial decontamination which essentially means to free of contamination. This can be interpreted to have the same meaning as sterilization and the results may sometimes be the same. However, in practice decontamination has the connotation of eliminating or destroying most microorganisms without attaining sterility.

The terms microbial decontamination, sterility and sterilization will appear frequently in discussions of spacecraft sterilization and it is important to recognize and understand the differences between the terms. There are other terms used in this general area and although not as frequent as the foregoing terms, their use and meaning should be noted: Disinfection and antisepsis, are terms also denoting decontamination, but they will not be used for our purposes.

2. Analysis of Heat Sterilization

To understand sterilization processes, it is necessary to know something of the mathematical treatments used to analyze microbial population deaths due to sterilizing agents. When populations of microorganisms, primarily bacteria, are exposed to sterilizing influences, the number of viable cells decreases exponentially as a function of exposure time; the death of the population resembles the first order reaction law. Variations and exceptions to the logarithmic death rate do occur but in general the deviations are not marked so that the exponential concept is still a valuable tool for establishing guidelines to sterilization processes. The food canning and pharmaceutical industries have used the concept extensively to evaluate the resistance of bacteria to sterilizing agents.

For a given sterilization procedure, plotting the number of surviving bacterial cells on a logarithmic scale against time on a linear scale usually results in a good fit to a straight line. The resulting curve, termed a survivor curve, is described by the following equation:

$$K = \frac{1}{t} (\log N_o - \log N)$$

$$\text{derived from } N = N_o \times 10^{-kt}$$

where:

K = an experimentally determined constant.

t = time of exposure.

N_o = number of organisms viable at the beginning of time t.

N = number of organisms viable at time t.

Logarithms are to the base 10.

There are several factors which affect the value of K. These include the following: temperature, dose-rate, or concentration of the sterilizing agent; the species and strain of the microorganisms, whether the organisms occur as spores or vegetative cells, and the environmental history of the cells including various secondary environmental factors of the sterilization process. If the microbial population being sterilized is a mixed population of

various species and strains, the resistance of the population probably exhibits considerable variability so that the death rate will be complicated beyond the simple exponential function. The unknowns are sufficient to prohibit publication of "K-tables".

Another concept can be derived from the exponential death rate. If the death rate of a population of microorganisms is truly exponential, then equal fractions of the population are killed in equal time intervals. A 90 percent reduction in the population or conversely, a survival of 10 percent can be expressed in terms of K by the following equation:

$$\log N_o - \log N \text{ and } N = \frac{1}{10} N_o$$

$$\text{then } \log N_o - \log \frac{N_o}{10} = \log 10 = 1 \text{ and } K = \frac{1}{t}$$

In terms of time, t, the equation becomes

$$t = 1/K$$

This time is called the decimal reduction time, represented by "D" and is defined as the time required to destroy 90 percent of a microbial population with given sterilization conditions. In heat sterilization work a D value may be determined for a number of different temperatures and in these instances it is customary to use a subscript designation for the particular temperature used, such as D_{135}, D_{125}, etc.

3. Capsule Sterilization

a. Space Environment

Simulated deep space environments have been investigated for its sterilizing influence since its vacuum, temperature and radiation conditions create a harsh environment for life forms. Studies have been conducted to determine whether these conditions might produce sterility of a spacecraft. It is known from past studies of low temperature effects on microbial cells that a temperature as low as that of liquid nitrogen will not destroy all microorganisms. Hard vacuums of 10^{-9} to 10^{-11} Torr did not kill all bacterial and fungal cells after several days exposure. Lyophilization or drying under vacuum in a frozen state, is a standard laboratory method for preserving microorganisms. Studies have shown that the condition for sterilizing a spacecraft by space radiations, ionizing and ultraviolet, are inadequate, furthermore a sterilizing dose of ionizing radiation will degrade many materials used in spacecraft. In short, the space environment will not kill all microorganisms on exterior surfaces and has less effect on those cells imbedded in the interior of materials.

b. Location of Microbial Contamination

Before discussing sterilization methods, the location of microbial c[illegible] on the spacecraft should be examined. It is obvious that external su[illegible] and components may be contaminated with microorganisms because of exposure to contaminating sources. The existence of internal microbial contamination may not be as obvious. Internal contamination of piece-parts occurs from the sealing of contaminated materials into a part during the manufacturing process. Surface contamination contributes to the internal contamination of components and assemblies when contaminated surfaces are interfaced and sealed, or when surface contaminated parts are covered with potting compounds or conformal coatings. Gross surface contamination then can give rise to gross internal contamination, a much more difficult sterilization problem.

c. Decontamination Methods

The ideal sterilization method for space vehicles would be a single treatment yielding sterility of the totally assembled vehicle without degradation of functional reliability. This is the goal of the spacecraft sterilization research and development program. The sterilization methods which are available for the destruction of microbial life are chemical, radiation, and heat.

Chemical sterilization methods employ agents in the form of liquids, vapors, or gases. Their use is restricted primarily to surfaces as they are useful, but not sufficient when internal sterilization is also a requirement, as it is in spacecraft sterilization. Factors which determine the applicability of a chemical agent to spacecraft sterilization are:

1) Sporicidal activity
2) Reactivity with materials
3) Reliability
4) Subsurface penetration

Sporicidal activity is required since some of the microorganisms contaminating the spacecraft will certainly be resistant bacterial spores. Only a very few of the possible chemical agents are reliably sporicidal. Secondly, it is obvious that the agent must be non-destructive to the materials composing the spacecraft. Since the nature of sterilizing agents requires considerable reactivity most of those commonly used in microbicides are reactive to some degree with some materials. In addition, many chemical agents must be applied as solutions and the solvent may have a detrimental effect on the material to which it is applied.

Lack of reliability militates strongly against reliance on any chemical sterilant, in fact, as will be noted below, even the best of the chemical agents which are available are

subject to unpredictable variations in efficiency. The effect of such variations is sufficiently severe and is sufficiently unpredictable that we must and will henceforth refer to chemical agents not as sterilants, but rather as decontaminants.

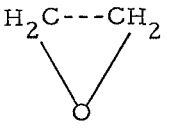
(1) Liquid Decontamination

Lack of penetration particularly restricts the use of liquid decontaminants; because of the greater or lesser surface tension inherent in any liquid microorganisms located in cracks, crevices and cavities may escape exposure to the decontaminating agent. However, liquid agents may still be useful in restricted, carefully controlled applications. Specifically, liquid agents have been studied for use in the decontamination and even sterilization of mating surfaces, lubricants and various fluids which may be carried on the spacecraft. Such applications however, are not contemplated for spacecraft presently under consideration. At the present state-of-the-art, a number of different liquid decontaminating agents would be required to be effective in the various possible applications and yet to avoid degradative reactions with the materials of the spacecraft. The present requirements for very detailed and specific use procedures and monitoring procedures would result in practice in an untenable compromise of reliability. A final problem in the use of liquid decontaminants is the fact that they tend to leave deposits both of the active material if it was a solid and of particulate matter carried in the liquid which is concentrated by evaporation of the liquid possibly interfering with the heat conduction or electrical properties of the surface.

(2) Gaseous Decontamination

A gaseous or vapor phase decontaminating agent is desirable for use on an item such as a spacecraft which has a great variability in surface configuration. Three such agents that have been investigated extensively for use on spacecraft are: ethylene oxide, formaldehyde and β propiolactone. In rating the sporocidal activity of these chemicals in the vapor phase, formaldehyde is about 150 times and β propiolactone about 4000 times more sporocidal than ethylene oxide for the same exposure conditions. However, both of the latter agents have undesirable properties; their vapors do not penetrate readily into porous materials or areas with restricted openings; both require relative humidities of 70 percent or higher to be efficient as sporicides; both tend to polymerize and leave residues which may interfere with component function. These properties make formaldehyde and β propiolactone unsatisfactory for any extensive use in decontamination procedures for spacecraft.

The third agent, ethylene oxide, (ETO) does not have the objectionable properties the other two have. This chemical is a cyclic ether with the following structural formula:



Its boiling point is 10.7°C (51.3°F) so that under ordinary conditions of temperature and pressure it is a gas. Liquid ethylene oxide is generally stable to detonating agents, however, mixed with air the explosive limits are generally cited as 3 to 100 percent by volume. The explosive hazard can be eliminated by proper mixture of ethylene oxide with inert gases such as carbon dioxide or dichlorodifluoromethane (Freon 12). The ETO/Freon mixtures have a very high dependence of concentration with pressure. This characteristic makes it possible to attain higher concentrations of ethylene oxide under restricted pressure conditions. The mixture that has been specified for use with spacecraft is 12 percent ethylene oxide and 88 percent dichlorodifluoromethane by weight.

In the gaseous state and under proper conditions of use, the ethylene oxide mixture is relatively inert to most materials. However, certain problem areas do exist. The vapor is absorbed by some plastic materials and may produce "crazing" a condition referring to a wide variety of physical alterations of the plastic. For example, acrylic and especially polystyrene plastics are subject to crazing by exposure to ethylene oxide. This crazing may not affect the strength and general configuration of the plastic but may disturb the optical properties. Therefore, optical systems made of Lucite or Plexiglass should not be exposed to ethylene oxide decontamination processing without adequate quality assurance test procedures. Ethylene oxide is also strongly absorbed by neoprene and other rubber materials causing swelling when they are exposed to the gas. The absorbed ethylene oxide is released from the materials within a few hours after the end of exposure and no permanent degradation results.

Ethylene oxide has very good diffusion properties, a desirable characteristic from the decontamination view point since the gas will diffuse into small cracks, crevices and porous materials. It also diffuses readily through a number of the plastic films such as polyethylene and polyvinyl although only slowly through dense films such as Mylar. The property of diffusion can be damaging, however, if a vacuum is pulled on a piece of equipment, assembly, or spacecraft immediately after ETO decontamination. Gas which had diffused into materials or through minute apertrues may not escape rapidly and may expand under vacuum possibly causing separations or distortion of the affected parts.

The microbicidal activity of ETO is dependent upon its concentration, temperature, exposure time, humidity and interrelationships of the variables. In addition, the resistance of the microbial population being exposed to the gas must also be considered. For ETO to be an effective microbicide the proper conditions must be present at the site of the microbial cell during the exposure period. With sufficient experimentation, it is possible to establish the proper exposure conditions for processing relatively simple materials to produce sterility but even with the use of organisms of known resistance "skips" or lack of sterility appear. To produce the proper conditions at all sites on a complex piece of equipment such as a spacecraft is extremely difficult. There are problems of diffusion, absorption, phase

distribution, adequate temperature, and perhaps condensation. Because of the several dependent variables involved, it is impossible to specify ETO as a sterilizing agent for spacecraft. Therefore, as mentioned above it is specified as a decontaminating agent for its use in the spacecraft sterilization program.

d. Sterilization Methods

The heat sterilization process described in Paragraph IV. B. 2. is the only method at the present state of the art which can yield sterilization. Due to the hardware requirements the heat cycle must be dry and this is accomplished through the use of hot dry nitrogen purging. For complete details on this method see the JPL Specification XSO-30275-TST-A.

C. CONCEPTS OF ASSAY AND CERTIFICATION

1. Scope

This paragraph outlines an assay and certification program to implement the sterilization requirement imposed on planetary-impacting or orbiting spacecraft. A program of standardized techniques and procedures is necessary for monitoring any facility involved in the assembly and/or testing of hardware that is required to be sterile. The overall purpose of this program is to provide the evidence necessary for certification of hardware sterility.

2. Certification of Sterility

a. Sources of Microbial Contamination

In general, three sources of microbial contamination may be identified within any facility involved in assembly and/or testing of spacecraft, namely, the animate beings operating the facility, the air entering and recirculating throughout the facility, and the hardware, tools and other inanimate objects necessary to operate the facility. Furthermore, operating personnel are usually considered the primary source of microbial contamination within a given enclosure, and, under specific conditions, the intramural environment of such an enclosure might constitute a reservoir of microbial contamination.

b. Control of Microbial Contamination

The personnel, and environmental requirements (air filtration, material decontamination or sterilization, tool decontamination or sterilization, etc.) imposed by the Interim Requirements for Bioclean Facilities were designed to reduce and control dissemination of microorganisms by clean room personnel during spacecraft assembly and/or testing.

3. Outline of Monitoring Methods to Provide Documentation for Certification of Spacecraft Sterility

a. Methods of Assay

1) Microbiological Assays

a) Microbiological sampling of intramural air of facility

(1) Continuous air sampling (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

(2) Grab samples (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

(3) Sampling and assaying procedure (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

b) Microbiological sampling of environmental surfaces

(1) Microbial fallout sampling surface

(a) Stainless steel strips (1.0 by 3.0 by 0.06 in.)

(2) Sampling and assaying procedure (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

c) Microbiological sampling of spacecraft hardware

(1) Surfaces

(2) Interiors

(3) Sampling and assaying procedure (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

d) Microbiological sampling of clothing and packaging materials

(1) Sampling and assaying (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

e) Microbiological sampling of personnel

(1) Sampling and assaying (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

b. Total Airborne Particle Count

(1) Sampling and assaying (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

c. Visual Assay (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

d. Parametric Measurements

(1) Permanent records
  (a) Facility temperature
  (b) Facility pressure
  (c) Facility relative humidity
(2) Other documentation (see JPL Specification GMO-50470-GEN and JPL Sterilization Group Procedure No. 1)

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4. Rationale

The microbiological assaying methods and the other monitoring procedures outlined in this section are aimed at the specific purpose of determining the level of microbial contamination within the facility and also in and on the spacecraft (see JPL Specification GMO-50470-GEN). The above mentioned procedures also serve as control measures by detecting the occurrence of "breaks-in-technique" or other atypical situations that might accidentally increase the microbial load in or on the spacecraft or spacecraft hardware. The air sampling procedures described have been used quite extensively and have proven reliable in measuring the level of microbial contamination per given volume of air. The Rodac plate has been proven reliable as a method of sampling surfaces. Microbial fallout sampling strips are being used more extensively in several geographical areas of the United States. Use of sterile strips, placed in close proximity to spacecraft hardware, as collecting surfaces to measure the degree of microbial fallout and accumulation is logical. Experimental evidence to date does not negate use of such strips, indeed more work is necessary to confirm and explain an apparent plateau that appears to develop during the first week of strip exposure and remain within a range of 10^3 (1,000 to 9,999) viable microorganisms per square foot over exposure periods up to one year.

At present, the weakest link in the monitoring chain appears to be the assaying of microbial contamination within and on spacecraft hardware. Specific procedures such as ultrasonic cleaning, vapor degreasing, and washing may provide some mechanical removal of surface microbial contamination. Exposure to ETO vapor tends to reduce surface microbial contamination, however, spacecraft hardware compatibility tests state a specified number of exposures to ETO, thereby emphasizing the importance of the parametric measurement approach to the overall program of monitoring. Thus, the above physical and chemical procedures, supplemented with microbiological sampling, are all aimed at reducing the level of surface microbial contamination and maintaining a specified low level of contamination throughout assembly. At present, the only reliable method of reducing or destroying microbial contamination within and on spacecraft parts is dry heat. All other methods should be regarded as decontamination procedures.

The results obtained and the records kept for all monitoring operations should provide a reasonable estimation of the level of microbial contamination present within the facility and on the spacecraft hardware at specific intervals. Also, cumulative or accidental increases in the microbial load of the spacecraft may be detected during assembly and/or testing and corrective decontamination and control procedures may be initiated. The data obtained during the entire program of monitoring may be used directly, or it may be incorporated into a mathematical logic network, to provide documentation that, prior to the terminal sterilization cycle with dry heat, the microbial load on any given spacecraft was at a minimum acceptable level, and that all of the personnel and environment control constraints necessary to achieve and maintain low levels of microbial contamination had been implemented throughout assembly and test of the spacecraft awaiting terminal dry heat sterilization.

However, the monitoring operation does not terminate prior to the terminal dry heat sterilization cycle. Indeed, the final phases of monitoring temperature will make use of thermocouples placed throughout the spacecraft to include thermally isolated areas. The majority of these thermocouples will not be retrievable. Detailed records are necessary to insure that the entire spacecraft was exposed to a temperature of 135°C for a 24 hours period. During development of equipment and also standardization of terminal dry heat sterilization procedures it will be mandatory to include the biological dry heat sterility test system developed under contract with Wilmot-Castle Company by the Jet Propulsion Laboratory.

Taken collectively, the above listed procedures represent the present state-of-the-art and knowledge in bioclean room monitoring. It must be realized that these procedures are subject to updating upon development of new procedures or acquisition of more knowledge. In fact, the approach to assay and certification as presented here, is a working model. As inputs from the JPL/Douglas study, Projects EASL and other studies become available, this document shall be reviewed and updated where indicated.

It is realized that this approach requires a sophisticated system of data recording, retrieving and analysis. The subject of data handling has been reviewed in Section III of this EPD.